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Water quality management in freshwater aquarium through submerged aquatic macrophytes (*Hydrilla verticillata* and *Vallisneria americana*) and their effects on servility of *Labeo rohita* fingerlings

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Abstract — *The health and survival of fish in closed systems like aquariums are heavily influenced by water quality. A study conducted from January to May 2023 at Vidyasagar University, West Bengal, aimed to evaluate the impact of two submerged aquatic macrophytes, Hydrilla verticillata and Vallisneria americana, on the water quality and survivability of Labeo rohita fingerlings in freshwater aquariums without artificial aeration. The experiment involved five treatment groups: low and high densities of Hydrilla (T1, T2), low and high densities of Vallisneria (T3, T4), and a mixed plantation of both species (T5). Each aquarium contained an equal number of L. rohita fingerlings, all managed under identical conditions. Water quality parameters, including temperature, dissolved oxygen (DO), pH, total alkalinity, total hardness, ammonia, nitrite, and nitrate, were monitored monthly. The findings revealed treatment-specific variations in water quality, with all parameters remaining within acceptable limits for freshwater fish culture. Notably, dissolved oxygen levels decreased as water temperature increased, while ammonia and nitrite concentrations remained below harmful thresholds. Nitrate levels varied across treatments, reflecting changes in nutrient dynamics linked to the different macrophyte species. The highest fish survivorship (100%) was observed in the high-density Vallisneria treatment (T4), while the high-density Hydrilla treatment (T2) recorded the lowest survival rate (50%). These results indicate that submerged aquatic macrophytes can effectively enhance water quality in aquariums without mechanical aeration, with V. americana showing particular promise as a sustainable, eco-friendly option for freshwater aquarium management.*

Keywords*— *Aquarium management, Water quality, Labeo rohita, Aquatic macrophytes, Vallisneria.

I. INTRODUCTION

Water quality is one of the most important aspects affecting the productivity, health, growth, physiological performance and survival of cultured fish in freshwater aquaculture systems. In closed systems, water exchange is limited and biological loading grows progressively, so ideal physicochemical conditions must be maintained to ensure fish welfare and ecological stability (Boyd & Tucker, 2012; Wetzel, 2001). Water quality consists of several interrelated physical and chemical parameters such as temperature, dissolved oxygen (DO), pH, total alkalinity, total hardness, ammonia, nitrite and nitrate which are directly related to fish metabolism, immune competence, feed utilization and overall physiological performance (Bhatnagar & Devi, 2013; El-Sayed, 2020). Even slight variations outside the optimal range may lead to chronic stress, disrupt osmoregulation, weaken immunological responses, decrease growth, and increase disease susceptibility, finally impacting fish survival and production efficiency (Boyd & Tucker, 2012; FAO, 2022).

Dissolved oxygen is one of the most important indicators of water quality among the factors as it influences respiration, aerobic metabolism and energy production in fish. High stocking densities, breakdown of organic matter and microbial respiration typically create oxygen deprivation in intensive growth systems (Boyd & Tucker, 2012). Water temperature is also a primary factor influencing metabolic rate, oxygen requirement, enzyme activity, and the toxicity of dissolved metabolites. As a rule, higher temperatures decrease the solubility of oxygen, and increase microbial breakdown and fish metabolism, consequently raising physiological stress (Wetzel, 2001; El-Sayed, 2020). Therefore, temperature and dissolved oxygen interactions are major factors influencing fish performance in confined aquatic systems. Nitrogenous metabolites are also as important water quality indicators. In aquaculture systems, ammonia is considered as the most harmful compound and is mostly generated via fish excretion and degradation of uneaten feed. Elevated ammonia concentrations cause impaired gill function, decreased oxygen intake, altered acid–base balance and reduced immunological function (Colt, 2006). Microbial nitrification is the oxidation of ammonia to nitrite and then to nitrate. Nitrate is much less toxic compared to ammonia or nitrite but long-term accumulation can have a negative effect on the growth of fish and the quality of water, particularly in recirculating or closed systems (Timmons & Ebeling, 2013). Hence, effective regulation of nitrogen cycling is a fundamental goal in sustainable aquaculture.

Apart from nitrogenous chemicals, pH, alkalinity and hardness together determine the chemical stability of freshwater habitats. Water pH influences food availability, enzyme activity, and toxic effect of dissolved compounds . Alkalinity provides buffering capacity that decreases pH changes that can be very fast due to biological activities such as respiration and nitrification (Boyd & Tucker, 2012). Total hardness, which is principally contributed by calcium and magnesium ions, is implicated in osmoregulation, skeletal development and physiological homeostasis in freshwater fish (Bhatnagar & Devi, 2013). Therefore, keeping these parameters within proper ranges is crucial to reduce the environmental stress and support the healthy growth of fish. The usual methods of aquarium management depend on artificial aeration, mechanical filtration and large water changes to keep the water in acceptable condition. These methods are effective but they also increase operational cost, use electrical energy and require regular maintenance making them less sustainable for low input aquaculture systems (Timmons & Ebeling, 2013). This has led to a heightened focus on eco-friendly alternatives that utilize natural biological processes to enhance water quality and reduce reliance on energy-intensive technologies (FAO, 2022). Recent advances in ecological aquaculture have focused on the utilization of aquatic macrophytes as natural biofilters to enhance water quality by assimilating nutrients, producing oxygen, stabilizing sediments, and promoting microbial-mediated nutrient cycling (Jeppesen *et al.*, 2012; Vymazal, 2013). The ecosystem services provide a viable basis for the development of sustainable management techniques for aquariums integrating biological components into water purification systems.

In recent years, submerged aquatic macrophytes have attracted increased attention as natural biological filters due to their potential to improve water quality and improve ecosystem functioning. In contrast to conventional filtration systems that require external energy inputs, submerged plants control water quality through nutrient uptake, photosynthetic oxygen release, sediment stabilization and interactions with microbial communities (Jeppesen *et al.*, 2012; Bornette & Puijalon, 2011). These ecological functions help in maintaining the physicochemical stability of the freshwater ecosystems and have been successfully used in ecological restoration, built wetlands, integrated aquaculture and recirculating aquatic systems (Vymazal, 2013; Madsen *et al.*, 2001). Therefore, the addition of submerged macrophytes in aquaria has been suggested as an environmentally sustainable way to decrease the accumulation of nutrients, reduce the use of mechanical filtration and the frequency of water replenishment. Dissolved nutrients can be removed by aquatic macrophytes by direct uptake from the water column and the sediment. The nitrogen and phosphorus taken up by plant tissues are then integrated into new biomass, limiting the availability of nutrients for undesired algal development and improving water clarity (Wetzel, 2001; Carpenter & Lodge, 1986). In addition, submerged vegetation provides large surface areas for colonization by nitrifying and denitrifying bacteria that facilitate the biological conversion of harmful ammonia to nitrite and then to less toxic nitrate (Kadlec & Wallace, 2009). The overall activity of plants and related microbial biofilms thus improves the efficacy of biological filtration and provides long-term ecological stability in closed aquatic habitats (Jeppesen *et al*., 2012).

Hydrilla verticillata (L.f.) Royle, a submerged freshwater macrophyte, has been well known for its extraordinary growth rate, adaptability and ability to extract dissolved nutrients from aquatic habitats. The species has a high rate of vegetative propagation, a high photosynthetic efficiency and a remarkable tolerance to a wide range of environmental conditions and therefore is able to colonize a variety of freshwater habitats throughout the world (Langeland, 1996). Due to its rapid growth, *H. verticillata* can quickly absorb dissolved inorganic nitrogen and phosphorus and decrease nutrient concentrations in the water around (Carpenter & Lodge, 1986). Dense stands of Hydrilla also produce large amounts of oxygen during daylight via photosynthesis, therefore increasing aerobic conditions for aquatic species and enhancing microbial-mediated nutrient cycling (Haller, 1974). However, a very high amount of plant biomass may lead to higher respiration at night and localized oxygen depletion, demonstrating that plant density is a significant determinant for the overall ecological performance (Langeland, 1996). *Vallisneria americana* Michx. is another widely distributed submerged macrophyte and is morphologically and ecologically different from Hydrilla. The species has long, ribbon-like leaves originating from basal rosettes and a large root system that efficiently absorbs nutrients from silt and stabilizes the substrate (Korschgen & Green, 1988). Vallisneria, in contrast to Hydrilla, typically supports an open underwater structure that allows water circulation and offers structurally complex habitat for fish and aquatic invertebrates (Catling et al., 1994). Previous studies have demonstrated that Vallisneria meadows host a diversity of microbial communities, contribute to nutrient cycling, and promote water clarity by minimizing sediment resuspension (Bornette & Puijalon, 2011). These ecological characteristics imply that *V. americana* may simultaneously enhance water quality and provide suitable environmental conditions that alleviate physiological stress in cultured fish. Submerged macrophytes, beyond their physicochemical roles, contribute to the biological integrity of freshwater ecosystems, enhancing habitat heterogeneity and biodiversity. Aquatic vegetation offers sanctuary from aggressive interactions, adds surface area for periphyton to grow, and generates microhabitats to sustain advantageous microbial populations for decomposition and nutrient transformation (Savino & Stein, 1982; Jeppesen *et al.*, 2012). Such ecological services are especially important in the confined aquarium systems where the environmental complexity is naturally limited. Aquatic plants are related with reduced behavioral stress, greater eating efficiency and improved welfare in a variety of freshwater fish (Bornette & Puijalon, 2011). Although numerous studies have shown the ecological importance of submerged aquatic vegetation in natural lakes and wetland ecosystems, relatively few studies have systematically compared different submerged macrophyte species in controlled aquarium conditions, particularly in systems maintained without artificial aeration. Previous studies have mainly focused on nutrient removal efficiency, aquatic ecosystem restoration or constructed wetlands, while information on the combined effect of macrophyte species and planting density on water quality dynamics and fish survivability in freshwater aquarium systems is relatively scarce (Vymazal, 2013; Jeppesen *et al*., 2012). Further studies are needed to analyses the relative efficacy of diverse submerged aquatic plants as biological components for sustainable aquarium management.

The Indian large carp *Labeo rohita* (Hamilton, 1822) is one of the most economically important freshwater fish species cultured throughout South Asia and particularly in India, Bangladesh, Nepal and Pakistan.

Because of its fast growth rate, good market acceptance, high nutritional value and compatibility with polyculture systems. *L. rohita* makes a significant contribution to freshwater aquaculture production and rural livelihoods (FAO, 2022; Jena & Das, 2011). Traditionally the species is cultured in earthen ponds but the increasing interest in indoor fish keeping, broodstock maintenance, educational aquaria and experimental research has highlighted the need for the development of biologically stable aquarium systems that can maintain favorable water quality without the need for constant mechanical intervention. Several research have examined the effects of water quality on the growth, physiology and health of *L. rohita* under pond and intensive culture conditions (Boyd & Tucker, 2012; El-Sayed, 2020). However, comparatively little attention has been given to the use of submerged aquatic macrophytes as natural biological filtration systems for the maintenance of water quality in freshwater aquaria containing this economically important carp. Previous studies have mainly concentrated on recirculating aquaculture systems (RAS), biofilters, constructed wetlands or integrated aquaculture, but relatively few studies have evaluated the performance of submerged macrophytes in closed aquarium conditions without artificial aeration (Timmons & Ebeling, 2013; FAO, 2022). Therefore, the practical usefulness of submerged aquatic vegetation as a green component of management of freshwater aquaria is still not well recognized. Plant species vary widely in shape, growth habit, nutrient uptake method and ecological role, any or all of which may affect their effectiveness in regulating water quality. Similarly, planting density may affect photosynthetic oxygen production, capacity for nutrient uptake, microbial colonization, and habitat complexity. *Hydrilla verticillata* and *Vallisneria americana* are two of the most widely distributed submerged freshwater macrophytes however there are few direct studies of their performance under similar aquarium settings . Also, nothing is known about the effect of varying planting densities on dynamics of water quality and survivability of fish. Knowledge of these distinctions is critical for the selection of appropriate macrophyte species for sustainable management of aquaria and low-input aquaculture systems. Beside increasing the physicochemical water quality, submerged macrophytes can also affect fish performance through ecological pathways independent of nutrient removal. Aquatic vegetation offers refuge from stress, enhances habitat diversity, modulates fish behavior, maintains periphytic microbial communities, and contributes to higher ecological resilience (Savino & Stein, 1982; Bornette & Puijalon, 2011). These biological interactions may be of particular importance in closed systems of aquariums, where fish are constantly exposed to changes in the environment and limited habitat. Therefore, the assessment of both water quality and fish survivability offers a more comprehensive assessment of the ecological effectiveness of submerged aquatic vegetation than an assessment of physicochemical parameters alone. While many studies have documented the role of aquatic vegetation in lake restoration and wastewater treatment, limited studies have concurrently evaluated water quality dynamics and fish survival under various submerged macrophyte compositions in controlled freshwater aquaria. Besides, the literature does not provide comparable data on *Hydrilla verticillata* and *Vallisneria americana* in non-aerated aquarium circumstances. This study was undertaken to experimentally evaluate the capacity of these two submerged macrophyte species to maintain adequate physicochemical conditions for freshwater fish under controlled laboratory circumstances, filling this gap of knowledge.

The present study aimed at (i) evaluating the efficacy of *Hydrilla verticillata* and *Vallisneria americana* at varying planting densities in maintaining water quality of freshwater aquaria without artificial aeration; (ii) comparing the temporal variations in the major physicochemical water quality parameters viz. water temperature, dissolved oxygen, pH, total alkalinity, total hardness, ammonia, nitrite and nitrate among different macrophyte treatments; and (iii) assessing the effect of different submerged macrophyte compositions on the survivability of *Labeo rohita* fingerlings. The results are expected to assist in developing environmentally sustainable and low-cost biological approaches for freshwater aquarium management and to provide useful baseline information for future studies on plant-based water quality management in aquaculture systems.

II. MATERIALS AND METHODS

2.1. Study site

The experiment was performed in the Department of Fishery Sciences, Vidyasagar University, Midnapore, West Bengal, India. The investigation was conducted during five consecutive months from January to May 2023 under controlled indoor laboratory settings. To minimize external variance, the aquaria were maintained under same ambient conditions throughout the experiment.

2.2. Experimental setup

Table 1: Fauna and Floral density in experimental tank.

TANK NUMBER / FISH AND MACROPHYTES	T1 (Low Density)	T2 (High Density)	T3 (Low Density)	T4 (High Density)	T5 (Mix)
Hydrilla verticillata	**5**	**10**	**-**	**-**	**5**
Vallisneria americana	**-**	**-**	**5**	**10**	**5**
***Labeo rohita* (Fingerlings)**	**10**	**10**	**10**	**10**	**10**

The influence of submerged aquatic macrophytes on the survivability of *Labeo rohita* fingerlings and water quality of aquarium was evaluated in five independent freshwater glass aquaria. The experimental treatments differed solely in the composition and density of aquatic macrophytes, whereas the quantity of supplied fish was same in all treatments. In each aquarium 10 healthy fingerlings of *Labeo rohita* were stocked and equal fish density was maintained in all treatment. No extra fish were added during the course of the experiment. Healthy fingerlings of *Labeo rohita* (Hamilton, 1822) of almost equal size were employed for the experiment. The fish were acclimatized to laboratory conditions prior to stocking to minimize handling stress and to assure a uniform physiological condition among treatments. Equal quantities of fish were placed in each tank. Two submerged freshwater macrophytes were selected based on their ecological value and reported nitrogen removal capacity as follows: *Hydrilla verticillata* (L.f.) Royle and *Vallisneria americana* Michx. Before stocking into the experimental aquaria, healthy and disease-free plants of similar size were selected. During the experimental period all aquaria were filled with fresh water and maintained under the same management conditions. The study of the natural contribution of submerged macrophytes to water quality management was possible due to the absence of artificial aeration and mechanical filtration. The fish were uniformly fed (6 % of body weight) according to regular laboratory management procedures. Excess feed and visible waste were eliminated as necessary to maintain hygiene and to reduce excess organic loading.

The experimental units were kept under the same environmental parameters, such as photoperiod and laboratory temperature, over the five months of the study.

Fig. 1: Aquariums preparation for the experiments

Fig. 2: Vallisneria americana

Fig. 3: Hydrilla verticillata

Fig. 4: Fingerlings of Labeo rohita

Fig. 5: Check Fish Mortality Rate

Fig. 6: Management of Aquarium

Fig. 7: Sample water collection for testing

Fig. 8: Low Density Hydrilla

Fig. 9: High Density Hydrilla

Fig. 10: Mix density of Vallisneria and Hydrilla

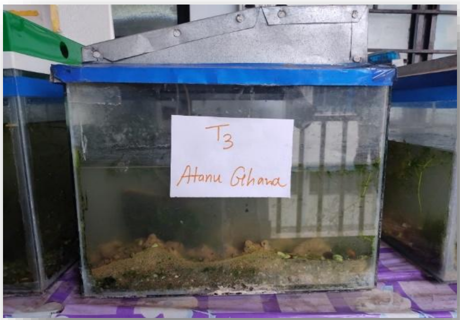

Fig. 11: Low Density Vallisneria americana

Fig. 12: High Density Vallisneria americana

Fig. 13: Tank Management

Fig. 14: Stocking of Hydrilla verticillata

Fig. 15: Water sample collection

2.3. Water quality assessment

Water quality was monitored monthly from January to May 2023. The following physicochemical parameters were determined: Temperature of water (°C), DO (mg L^{-1}), pH value, Total alkalinity (mg L^{-1} as $CaCO_3$), Total hardness (mg L^{-1} as $CaCO_3$), Ammonia (mg/L), Nitrate (mg L^{-1}), Nitrate (mg L^{-1}). Water quality was tested using commercial aquarium water testing kits (Transchem, WTK for Fisheries) and standard laboratory procedures as described in the original experimental protocol. Measurements were performed according to the techniques indicated by the manufacturer.

Fig. 16: Estimation of Dissolved oxygen

2.4. Survival of fish

Fish survival was evaluated at the end of the experiment by counting the number of live fish surviving in each aquarium. The percentage survival was assessed by: The observed survival percentages across the treatments were compared to evaluate the effect of aquatic macrophytes on the fish performance.

2.5. Statistical Analyses

Descriptive statistics were used to summaries the experimental observations. For all treatments, the monthly fluctuation of each physicochemical parameter was tabulated and treatment-wise averages were derived for comparison and Microsoft Excel (2010) was used for other statistical analysis.

III. RESULTS

Table 2: Water quality parameters of Tank-1, Low density of Hydrilla verticillata

WATER QUALITY PARAMETERS	MONTH 25.01.23	MONTH 24.02.23	MONTH 25.03.23	MONTH 24.04.23	MONTH 26.05.23	STANDARD DEVIATION
TEMPERATURE (°C)	18	23	25	30	32	± 5.595
DISSOLVED OXYGEN (PPM)	7.6	7.2	7.0	6.8	6.2	± 0.518
AMMONIA (PPM)	0.010	0.015	0.100	0.120	0.160	± 0.066
ALKALINITY (PPM)	55	62	80	92	100	± 19.162
NITRITE (PPM)	0.18	0.21	0.25	0.26	0.25	± 0.034
NITRATE (PPM)	0.05	5	10	13	15	± 6.090
PH	6.8	7.0	7.2	7.5	8.0	± 0.469
HARDNESS (PPM)	120	115	100	96	85	± 14.272

Table 3: Water quality parameters of Tank-2, High density of Hydrilla verticillata

WATER QUALITY PARAMETERS	MONTH 25.01.23	MONTH 24.02.23	MONTH 25.03.23	MONTH 24.04.23	MONTH 26.05.23	STANDARD DEVIATION
TEMPERATURE (^{O}C)	20	23	25	30	34	± 5.59464
DISSOLVED OXYGEN (PPM)	8.2	7.6	7.5	6.8	6.5	± 0.676018
AMMONIA (PPM)	0.018	0.02	0.12	0.11	0.13	± 0.055775
ALKALINITY (PPM)	50	68	75	100	125	± 29.27969
NITRITE (PPM)	0.185	0.192	0.2	0.21	0.23	± 0.017544
NITRATE (PPM)	0.2	0.5	1	1.2	2.5	± 0.88713
PH	6.5	7.2	7.4	7.5	7.8	± 0.486826
HARDNESS (PPM)	135	128	115	100	88	± 19.40876

Table 4: Water quality parameters of Tank-3, Low density of Vallisneria americana

WATER QUALITY PARAMETERS	MONTH 25.01.23	MONTH 24.02.23	MONTH 25.03.23	MONTH 24.04.23	MONTH 26.05.23	STANDARD DEVIATION
TEMPERATURE (^{O}C)	20	25	27	32	36	± 6.204837
DISSOLVED OXYGEN (PPM)	8.0	7.5	7.3	6.5	6.3	± 0.70852
AMMONIA (PPM)	0.02	0.08	0.13	0.145	0.18	± 0.06229
ALKALINITY (PPM)	45	70	88	110	135	± 34.83246
NITRITE (PPM)	0.12	0.16	0.2	0.215	0.22	± 0.042367
NITRATE (PPM)	1.5	2	2.8	3.2	4	± 0.984886
PH	7	7.2	7.5	7.7	8	± 0.396232
HARDNESS (PPM)	140	130	115	90	75	± 27.15695

Table 5: Water quality parameters of Tank-4, High density of Vallisneria americana

WATER QUALITY PARAMETERS	MONTH 25.01.23	MONTH 24.02.23	MONTH 25.03.23	MONTH 24.04.23	MONTH 26.05.23	STANDARD DEVIATION
TEMPERATURE (^{O}C)	22	25	28	30	35	± 4.949747
DISSOLVED OXYGEN (PPM)	8.2	7.6	7.3	6.8	6.0	± 0.831865
AMMONIA (PPM)	0.075	0.115	0.13	0.145	0.15	± 0.030125
ALKALINITY (PPM)	35	60	75	100	140	± 40.09364
NITRITE (PPM)	0.21	0.219	0.24	0.255	0.28	± 0.028119
NITRATE (PPM)	1.5	2.1	2.6	3	3.2	± 0.690652
PH	7.2	7	7.3	7.5	7.2	± 0.181659
HARDNESS (PPM)	145	126	110	90	65	± 31.0918

Table 6: Water quality parameters of Tank: 5, Mix density of Hydrilla verticillate and Vallisneria americana

WATER QUALITY PARAMETERS	MONTH 25.01.23	MONTH 24.02.23	MONTH 25.03.23	MONTH 24.04.23	MONTH 26.05.23	SD
TEMPERATURE (^{O}C)	20	26	30	32	35	± 5.813777
DISSOLVED OXYGEN (PPM)	6.4	5.8	5.6	5.4	5.2	± 0.460435
AMMONIA (PPM)	0.11	0.15	0.156	0.16	0.18	± 0.025636
ALKALINITY (PPM)	40	70	125	150	175	± 55.96874
NITRITE (PPM)	0.235	0.238	0.24	0.258	0.28	± 0.018926
NITRATE (PPM)	3	3.5	3.8	4	4.2	± 0.469042
PH	7	7.2	7.5	7.7	8	± 0.396232
HARDNESS (PPM)	145	120	110	85	55	± 34.3875

3.1. Temperature of water

Water temperature increased gradually across all experimental treatments over the five-month study period (January to May 2023). Temperatures reported ranged from 18 to 36 °C, which suggests the warming of seasons from winter to summer. Among the treatments, the lowest initial temperature (18°C) was observed in T1 (low-density *Hydrilla verticillata*) and the greatest final temperature (36°C) was observed in T3 (low-density *Vallisneria americana*). Mean water temperature ranged from 25.6 °C in T1 to 28.5 °C in T5 and was not significantly different among treatments.

3.2. Oxygen dissolved

Dissolved oxygen concentrations decreased progressively during the experimental period in all treatments. This reduction came when water temperatures increased. Average dissolved oxygen ranged from 5.68 to 7.32 mg L^{-1} . The maximum average dissolved oxygen content (7.32 mg L-1) was measured in T2 and the lowest average value (5.68 mg L-1) was observed in T5. However, dissolved oxygen remained within the permissible limit for freshwater fish culture throughout the investigation, even if there was a steady decrease.

3.3. Ammonia

Throughout the experiment ammonia concentrations stayed substantially below the critical threshold (<0.25 mg L^{-1}). However, an increase in ammonia concentration was detected in all aquaria over time. Lowest average ammonia content was recorded in T2 (0.079 mg L^{-1}) followed by T1 (0.081 mg L^{-1}). The greatest average of ammonia content was observed in T5 (0.150 mg L^{-1}). These results showed that nitrogenous waste gradually increased during the culture period but never reached the dangerous level to fingerlings of rohu.

3.4. Total alkalinity

Total alkalinity increased consistently during the whole trial period in all treatments.

The mean alkalinity ranged from 73.8 mg L^{-1} in T1 to 112 mg L^{-1} in T5 and falls within the acceptable range for freshwater farming. Alkalinity was highest in the mixed macrophyte treatment (T5) at all times throughout the trial.

3.5. Nitrite

Nitrite concentrations were low in all treatments. The average nitrite concentration ranged between 0.20 and 0.25 mg L^{-1} , demonstrating that nitrification processes were maintained during the experiment . The lowest average of nitrite concentration was in T2 and the greatest average of nitrite concentration was in T5.

3.6. Nitrates

The changes in nitrate buildup across treatments were pronounced. The minimum mean nitrate concentration was found in T2 (1.08 mg L^{-1}) while the maximum mean nitrate concentration was found in T1 (8.61 mg L^{-1}). T3, T4 and T5 showed intermediate levels of nitrate. However, all nitrate values were still much below the recommended maximum limit of nitrate concentration for freshwater aquarium systems .

3.7. pH, alkalinity and hardness

The pH of the water was rather steady during the research. The average pH values ranged from 7.24 to 7.48, which were near neutral to slightly alkaline conditions suited for cultivation of freshwater fish. The greatest mean pH was reported in T3 and T5 (7.48) while the lowest average pH was recorded in T4 (7.24).In all the treatments it was observed that water hardness gradually decreased during the trial period. Mean hardness values varied from 103.0 to 113.2 mg L^{-1}, within the desired range for freshwater aquaculture. T2 displayed the highest average hardness and T5 the lowest average hardness.

Differences across aquarium management practices were evident in the average of treatments. The high-density *Hydrilla verticillata* (T2) had the highest average dissolved oxygen concentration and the lowest quantities of ammonia, nitrite and nitrate, showing an efficient ability to regulate water quality. On the other hand, the mixed plantation (T5) showed relatively higher ammonia, alkalinity and nitrite concentrations with the lowest dissolved oxygen concentration. However, all water quality factors measured were within acceptable values for the culture of fresh water fish during the study period.

3.8. Fish survivability

Survival of fish varied significantly among treatments. The best survivability (100%) was found in T4 (high-density *Vallisneria americana*) and the lowest survivability (50%) in T2 (high-density *Hydrilla verticillata*). The survival rates were 70%, 60% and 60% in T1, T3 and T5 correspondingly. These results indicate that T2 offered a somewhat good physicochemical water quality, while the aquarium with high-density *Vallisneria americana* had the maximum fish survival. This shows that factors other than the measured water quality variables, such as habitat complexity or behavioral interactions, may also have affected fish survival in the experimental conditions. This is a hypothesis from the data we have collected, not a causal conclusion that has been demonstrated.

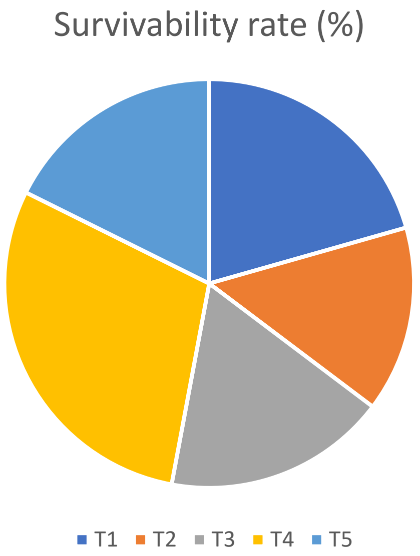

Fig. 17: Survivability rate of fingerling

Table 7: Fish survival at the end of the experiment

TREATMENT	FISH STOCKED	FISH SURVIVED	SURVIVAL (%)
T1	10	7	70
T2	10	5	50
T3	10	6	60
T4	10	10	100
T5	10	6	60

IV. DISCUSSION

4.1. Effect of submerged macrophytes on water quality in aquaria

The present study showed that the submerged macrophytes significantly influenced the physicochemical characteristics of aquarium water during the five months experimental period. Water quality of all treatments was within the permitted limits for freshwater fish culture. However, significant changes were noticed in the dissolved oxygen, nitrogenous compounds, alkalinity and fish survivorship. These results agree with previous studies in showing that submerged aquatic vegetation contributes to the functioning of aquatic ecosystems through assimilation of nutrients, oxygen production, sediment stabilization and stimulation of microbial processes (Carpenter & Lodge, 1986; Bornette & Puijalon, 2011; Jeppesen et al., 2012). Aquatic macrophytes are vital in maintaining ecological balance by controlling nutrient loading, increasing water transparency and providing refuge for microorganisms involved in nutrient cycling (Wetzel, 2001; Jeppesen et al., 2012). Similar positive benefits of submerged plants have been found in freshwater restoration and aquaculture systems (Vymazal 2013).

4.2. Water quality parameters

In all experimental aquaria, the temperature of the water climbed gradually from January to May, reflecting typical seasonal variation rather than effects of the treatments. All aquaria were maintained under the same environmental parameters; this trend was therefore expected.

Temperature is one of the most critical environmental parameters that influence fish metabolism and feeding behaviors, oxygen consumption, and microbial activity (Boyd & Tucker, 2012). The gradual decrease in dissolved oxygen seen during the experiment could potentially be due to the fact that the solubility of oxygen in water decreases as the temperature increases (Wetzel, 2001). Boyd and Tucker (2012), Bhatnagar and Devi (2013) have noted similar seasonal trends of temperature in freshwater aquaculture systems. The dissolved oxygen contents were steadily dropped over the investigation although they were in the permitted range for freshwater fish culture. T2 had the highest concentration of average dissolved oxygen among all treatments. This observation may be due to the fast photosynthetic activity of *Hydrilla verticillata* which is known to be one of the most productive submerged aquatic macrophytes (Langeland, 1996; Haller, 1974). Previous research has shown that dense beds of Hydrilla enhance oxygen production during the day and provide habitat for microbial communities associated with nutrient transformation (Carpenter & Lodge, 1986; Jeppesen et al., 2012). At night, however, submerged plants also respire and need oxygen. This may result in monthly sampling underestimating the diurnal oxygen dynamics. Wetzel (2001) and Boyd and Tucker (2012) have documented similar changes in dissolved oxygen related to aquatic vegetation. Ammonia, nitrite and nitrate concentrations were kept below threshold levels over the testing period demonstrating that the planted aquarium systems-maintained nitrogen cycling successfully. The reduced content of ammonia and nitrate in T2 is a reflection of the good digestion of nutrients by *Hydrilla verticillata*. Submerged macrophytes have been shown to soak up nutrients rapidly in freshwater settings (Carpenter & Lodge, 1986; Vymazal, 2013). Submerged plants take up nutrients directly and also provide large surface areas for nitrifying bacteria, which enhance biological filtration through coupled plant–microbial interactions (Wetzel, 2001; Kadlec & Wallace, 2009). T1 showed relatively higher nitrate accumulation compared to other treatments although all the measured amounts were much below the threshold suggested for freshwater fish rearing (Boyd & Tucker, 2012). Therefore, fish mortality was probably not directly caused by nitrate accumulation. The pH remained reasonably constant in all treatments, suggesting that sufficient buffering capacity was maintained during the experimental time. Total alkalinity increased gradually in all treatments, especially in mixed plantation aquarium (T5). This rise probably represents continual mineralization of organic materials coupled with carbonate buffering activities within the aquarium substrate (Boyd & Tucker, 2012). Adequate alkalinity is also important as nitrifying bacteria absorb bicarbonate ions during ammonia oxidation and consequently affect pH stability (Wetzel, 2001).

Total hardness was also within the approved limit for fresh water aquaculture. Calcium and magnesium ions are essential for osmoregulation, skeletal growth, enzyme activation and stress resistance in freshwater fishes (Boyd & Tucker, 2012; Bhatnagar & Devi, 2013).

4.3. Survivability of fish

The most interesting result of the present investigation was the significant difference in fish survival among treatments. T2 had relatively better water quality yet survivability of fish was just 50% as compared to 100% survivability in T4 despite the fact that T4 had only intermediate water quality. This is indicative that disparities in fish survival could not be explained by physicochemical water quality alone. One possible explanation is the difference in growth morphology of the two submerged macrophytes. *Vallisneria americana* has long ribbon-like leaves providing vertical organized habitat, shelter and open swimming corridors for fish (Korschgen & Green, 1988; Catling *et al.*, 1994). In contrast, dense Hydrilla creates tight branching vegetation which may impede water circulation and swimming space, while increasing nocturnal respiration within dense plant stands (Langeland, 1996). Another probable cause is changes in microbial colonization. Aquatic macrophytes have been shown to sustain specific bacterial populations involved in nitrogen transformation and pathogen control (Jeppesen et al., 2012; Vymazal, 2013). Because microbial community composition was not explored in the present study, this theory is speculative and needs additional exploration. Behavioral interactions among fish may also have differed among treatments, as habitat complexity strongly influences stress responses, predator avoidance behaviors and energy expenditure in fish (Savino & Stein, 1982). The greater survival seen in T4 may therefore be due to ecological benefits of Vallisneria habitat, and not only to water chemistry.

The present results are in line with the earlier publications showing that submerged macrophytes improve water quality by removal of dissolved nutrients and enhancement of biological filtration (Carpenter & Lodge, 1986; Jeppesen *et al.*, 2012; Vymazal, 2013). Many studies have indicated decreases in ammonia and increase in dissolved oxygen after the introduction of submerged vegetation into freshwater systems (Wetzel, 2001; Boyd & Tucker, 2012). However, unlike earlier research where increased water quality directly translated to higher fish performance, the present experiment showed that the treatment with the best physicochemical features did not result in the maximum survival. This shows that the architecture of the habitat, the behavioral ecology, the microbial interactions and any biological variables not examined may have a significant impact on the performance of the fish in stocked aquaria. Research on the ecological functions of submerged aquatic vegetation has shown similar conclusions (Bornette & Puijalon, 2011; Jeppesen *et al.*, 2012).

Table 8: Recommendation for aquarium water quality management

Purposes	**Macrophytes with their densities**
For better temperature management	Tank 4 (High density *Vallisneria americana)*
For better Dissolved Oxygen	Tank 4 (High density *Vallisneria americana)*
For lower ammonia rate	Tank 3 (High density *Hydrilla verticillata)*
For lower nitrite	Tank 2 (High density *Hydrilla verticillata)*
For higher nitrate	Tank 1 (Lower density of *Hydrilla verticillata)*
Optimal pH	Tank 3, Tank 4
Better alkalinity	Tank 3 (Low density of *Vallisneria americana)*
Better hardness	Tank 2 (High density of *Hydrilla verticilata)*

V. CONCLUSION

The present study showed that submerged macrophytes could maintain adequate water quality in freshwater aquaria without artificial aeration. The measured physicochemical parameters were in the range considered acceptable for fish culture in fresh water during the five months of the experiment. *Hydrilla verticillata* (T2) grown at high density seemed to have better control over dissolved oxygen and nitrogenous compounds. However, the highest fish survivability (100%) occurred in high-density *Vallisneria americana* (T4), while the lowest survival was in T2 (50%).This implies that fish survival was not solely a function of water physicochemical quality. The structure of *V. americana* could have provided better habitat conditions and less stress from the environment for the *Labeo rohita* fingerlings. In general, dense *V. americana* seems to be a promising candidate for an ecological approach to the sustainable low-input management of aquaria. Further studies including replication, behavioral observations and microbial-community analysis are recommended to elucidate the mechanisms responsible for the observed differences in fish survivability.

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CONFLICT OF INTREST

The authors declare that there is no conflict of interest.

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